REMARKS

Status of the Claims

Claims 1 to 11 and 21 to 22 were acted upon by the Examiner. Claims 1 to 3, 9, and 21 to 22 have been amended. Claims 12 to 20 have been canceled. Claims 23 to 30 have been added. Claims 1 to 11 and 21 to 30 are pending in the application.

Support for Amendments to the Claims

Support for claims 1-3 and claims 24-26 can be found on page 7, lines 18-20 which recite that the precursor cells (which may be neural stem cells or neural progenitor cells) may be isolated from peripheral tissue with sensory receptors, specifically olfactory epithelium or tongue.

Support for claim 21 can be found on page 8, lines 14-16 and page 10, lines 14-18 which recites the claimed uses of the olfactory precursor cells or cells differentiated from those cells.

Support for the uses described in claim 22 is found in claim 22 as filed and on page 10, lines 19-20 and 25-28.

Support for claim 23 is found on page 8, lines 12-24.

Support for claims 27 and 28 can be found on page 32, lines 19-25 which recite neurons, astrocytes and oligodendrocytes differentiated from tongue precursor cells.

Support for claim 29 is found in original claim 21, page 8, lines 17-23, page 33, lines 17-20 and page 10, lines 3-4

and 9-14 which describe the claimed treatments, including treatments of Parkinsons's disease and multiple sclerosis.

In view of the amendments discussed above, the subject matter defined by various of the dependent claims has changed. Support for the subject matter claimed in these non-amended dependent claims is discussed below.

Support for claim 4 is found on page 14, lines 16-24 which describe the olfactory precursor cells as expressing glutamic acid-decarboxylase.

Support for claim 5 is found on page 14, lines 5-6 which state that the olfactory cells can be differentiated into neurons, astrocytes or oligodendrocytes.

Support for claims 6 and 7 is found on page 9, lines 6-7 which describe the olfactory precursor cells transfected with a heterologous gene encoding a trophic factor.

Support for claims 8-10 is found on page 9, lines 10-11 which describes neurons, astorcytes and oligodendrocytes differentiated from the precursor cells and page 8, lines 9-14 and page 18, lines 24-26 which describe cells differentiated from olfactory precursor cells that express neuronal markers and differentiate into dopaminergic neurons.

Support for claim 11 is found on page 9, lines 12-16 which describes precursor cells in a pharmaceutical composition.

The Restriction Requirement

In the Office Action, the Examiner required election of a single group of claims from the following groupings:

I. Claims 1 to 11, 21 and 22, drawing to precursor cells of a mammalian peripheral tissue;

II. Claims 12 and 13, drawing to a method of treatment; or

III. Claims 14 to 20, drawn to a method of isolating and purifying precursor cells.

During a conference by telephone with the Examiner held on March 10, 1998, applicants provisionally elected to prosecute the Claims of Group I (Claims 1 to 11 and 21 to 22). Applicants hereby affirm this election. The remaining claims, Claims 12 to 20, have been canceled. Applicants reserve the right, however, to prosecute the canceled claims in future divisional applications.

Summary of the Examiner's Rejections

The Examiner had required filing of a new Oath or Declaration. Applicants have enclosed a signed Declaration which identifies the application by the attorney docket number of 21,451-B USA and Express Mail Label No. EM605908814US. At the time the inventors signed and dated the Declaration, the application number was unknown. Applicants did not receive the Filing Receipt granting the filing date and presenting the application number until January 9, 1998, after the inventors signed and dated the Declaration. Given these facts and the identification of both the Declaration and the application as

filed by attorney docket number 21,451-B USA and Express Mail Label No. EM605908814US, applicants request respectfully that the Examiner withdraw the requirement for an executed Declaration bearing the Application Number and filing date.

Claims 1 to 7, 22, 21 and 22 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1, 3 to 11, 21 and 22 have been rejected under 35 U.S.C. §112, first paragraph, as lacking enablement for precursor cells from the peripheral tissue of any mammal.

Each of these rejections is discussed hereinbelow.

The §112, Second Paragraph, Rejection

Claims 1-7, 11, 21 and 22 have been rejected under 35 USC 112, second paragraph, as indefinite.

The Examiner rejected claim 1 as confusing for reciting "Isolated precursor cells of a mammal from peripheral tissues containing sensory receptors." Applicants amended claim 1 to describe isolated precursor cells from an olfactory epithelium of a mammal, and have presented claim 24 to define isolated precursor cells from a tongue of a mammal.

The Examiner rejected claim 9 as vague and indefinite as to what is meant by "the cells express neuronal markers and contain dopaminergic neurons." The precursor cells are differentiated into dopaminergic neurons. Accordingly,

applicants have amended claim 9 to state that "the cells express neuronal markers and comprise dopaminergic neurons."

The Examiner rejected claims 2-11, 21 and 22 for reciting "the cells of claim 1". The Examiner requested clarification of whether the cells of claim 1 are the precursor cells or the stem cells and progenitor cells. The amendments to these claims clarify that the cells of claim 1 referred to in dependent claims 2 to 11, 21 and 22 are isolated precursor cells from olfactory epithelium of a mammal. Claims 25-30 refer to the tongue precursor cells of claim 24.

The §112, First Paragraph, Rejection

Claims 1, 3-11, 21 and 22 stand rejected under 35 USC 112, first paragraph, because the specification, while enabling for precursor cells isolated from olfactory epithelium of a mammal (also referred to in this response as "olfactory precursor cells"), allegedly does not provide enablement for precursor cells from the peripheral tissues of any mammal.

Claims 1-11, 21 and 22 are amended to recite isolated precursor cells from olfactory epithelium which overcomes this objection. The claims are amended without prejudice to filing a continuation application directed to the canceled subject matter. New claim 23 recites precursor cells from olfactory epithelium and new claims 24 to 30 recite precursor cells from tongue.

The Examiner alleged that the cells of the olfactory epithelium differentiated into neurons and oligodendrocytes but not astrocytes (page 6 of Office Action). The application describes the differentiation of the olfactory precursor cells

into astrocytes. Example 2 ("Differentiating Precursor Cells Into Neurons, Astrocytes and Oligodendrocytes") describes differentiation of postnatal rat olfactory cells into neurons (GABAergic neurons and dopaminergic neurons), astrocytes and oligodendrocytes (see page 17, line 12, page 18, line 18, page 19 line 5). The differentiation of passaged cells into neurons, astrocytes and oligodendrocytes is described on page 19 at line 14. Inducing of human precursor cells to differentiate into neurons, astrocytes and oligodendrocytes is described on page 28 in Examples 13-15. The markers used to precisely identify neurons, astrocytes and oligodendrocytes are described on page 17, line 18. The application provides ample support for claim 5 which refers to astrocytes differentiated from the olfactory precursor cells.

The Examiner also alleged that it would require undue experimentation by a skilled artisan to make and/or use precursor cells from peripheral tissues. These claims now refer to olfactory precursor cells and precursor cells from a tongue (also referred to as "tongue precursor cells" in this response). The methods for isolating olfactory precursor cells are identified in Example 1. The physical and functional characteristics of the olfactory precursor cells and methods for differentiating the cells are described in Example 2, described above. Example 3 describes differentiation of adult mouse and adult rat olfactory cells into neurons and oligodendrocytes at page 20, line 21. In view of these examples, the other teachings in the application and the knowledge in the art, a skilled artisan would be able to make and/or use olfactory precursor cells without undue experimentation. Similar methods are used to isolate tongue precursor cells (Example 18, "Isolation of Precursor Cells from Other Peripheral Tissues"). The specification also teaches that the tongue precursor cells are passaged and differentiated into neurons, astrocytes and oligodendrocytes

using similar techniques to those used for the olfactory precursor cells (page 32, line 19). Therefore, the specification shows a skilled artisan how to isolate and use the tongue precursor cells (which can be neural stem cells or neural progenitor cells) as now recited in claims 24 -28. As is discussed below, the specification also shows a skilled artisan how to make and use the tongue precursor cells according to claims 29 and 30.

Claims 21 and 22 were rejected because the structural and functional characteristics of precursor cells of peripheral tissues and of neurons, astrocytes or oligodendrocytes into which they differentiate are not predictable. The Examiner alleged that the application and prior art do not enable the use of the precursor cells obtained from any peripheral tissue recited in claims 21 and 22. Claims 21 and 22 now refer to olfactory precursor cells and cells produced from the olfactory precursor cells. New claims 29 and 30 refer to tongue precursor cells and cells produced from the tongue precursor cells. The structural and functional characteristics of the olfactory precursor cells and tongue precursor cells are described in the application (see the Examples in the application that are referred to in the preceding paragraphs of this response). The structural and functional characteristics of the neurons, astrocytes and oligodendrocytes produced from these precursor cells are predictable. For decades, primary cultures of astrocytes, oligodendrocytes, and neurons have provided the basis for much of the scientific literature in neuroscience. Moreover, many publications now detail the generation of astrocytes, oligodendrocytes, and neurons from progenitor or stem cells isolated from the central nervous system. For example, Gritti, A. et al. (1996). "Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor." *Journal of Neuroscience*

16(3): 1091-1100 (copy enclosed) explains how to differentiate and use central nervous system cells. The source of the precursor cells in this application differs, but the ways to generate and use those cells is known.

The specification provides sufficient guidance to enable the use of olfactory precursor cells for treatment of diseases, disorders and abnormal physical states. Claims 21 and 23 now recite the use of the olfactory precursor cells to treat diseases, disorders or abnormal physical states including neurodegenerative disease or neurotrauma. Claim 23 recites the neurodegenerative diseases as Parkinson's disease and multiple sclerosis.

The Applicants have shown how to isolate, culture and differentiate the olfactory precursor cells (Examples 1-3 and 7). The Applicants have also shown how to use the cells in treatment of Parkinson's disease (Example 16, pages 28-32). In patients with this neurodegenerative disease, dopaminergic neurons die which leads to a progressive loss of motor function and death. Example 16 shows how to transplant the olfactory precursor cells into an animal model of Parkinson's disease in order to treat the disease by *in vivo* differentiation of the precursor cells into dopaminergic neurons. In this procedure, isolated olfactory cells were sterotactically injected into the rats. The cells then spontaneously differentiated into dopaminergic neurons without the addition of extrinsic chemicals. The presence of dopaminergic neurons was confirmed by identifying tyrosine hydroxylase positive (TH) neurons (Tyrosine hydroxylase is a marker for dopaminergic neurons (see page 22 line 6, page 20, line 27 and page 21, line 4)). Similar results may be obtained with the 6-OHDA animal model of Parkinson's disease (page 31, line 19). These working examples show that

precursor cells can be derived from biopsies of the olfactory epithelium of Parkinson's disease patients and used as an autologous source of neurons for transplantation (page 30, line 25).

These experiments also show that the olfactory precursor cells are useful for treatment of other neurodegenerative diseases or neurotrauma. The olfactory precursor cells are peripheral nervous system cells that can differentiate into cell types such as oligodendrocytes that are only found in the central nervous system (page 31, line 7). The cells therefore differentiate into cell types that are useful for treatment of a wide range of neurodegenerative diseases and neurotrauma.

The ultimate developmental outcome for the progeny of the olfactory precursor cells depends primarily on the local neural environment. Thus, these cells can be transplanted for treatment of diseases, disorders or abnormal physical states such as neurodegenerative disease and neurotrauma without the need for exogenous chemicals to direct the differentiation of the precursor cells. The transplantation techniques for treatment of other diseases are identical to those described in Example 16. Stereotactic injection of precursor cells into injured, abnormal or diseased neural tissue is performed and the precursor cells are permitted to spontaneously differentiate *in vivo* into healthy neurons, astrocytes or oligodendrocytes.

In summary, the injection technique for transplantation of the cells is simple. The differentiation of the transplanted cells is influenced solely by the local neural environment rather than by human manipulation of the cells before or after transplantation of the cells. As a result, no

undue experimentation is required to make or use the cells to treat neurotrauma or neurodegenerative diseases such as multiple sclerosis. A skilled person would clearly know that the claimed diseases, disorders and abnormal physical states could be treated using the olfactory precursor cells.

Tongue precursor cells are also useful for treatment of neurodegenerative diseases or neurotrauma, including Parkinson's disease and multiple sclerosis in a similar manner (example 18, page 33, line 16). The transplantation of the tongue precursor cells is performed by stereotactic injection which is the same technique used for transplantation of the olfactory cells.

The specification also discloses how the olfactory and tongue precursor cells may be transformed with a gene. Example 8 on page 23 describes transfection of olfactory precursor cells with vectors to introduce growth factors. On page 24, lipofectamine and adenovirus mediated transfection strategies of olfactory precursor cells are discussed. The protocols for transfection are described in references in the application on line 21 which are incorporated into the application by reference. In view of these references in the application, the application itself and the state of the art, strategies for carrying out transfection experiments would be apparent to a skilled artisan without undue experimentation. Example 10 provides a working example of transfection of olfactory precursor cells using the adenovirus gene system with ß-galactosidase as a marker gene (see Fig. 6 also) A person skilled in the art would easily be able to express other genes in the olfactory precursor cells using similar techniques. A skilled person would know what other genes could be expressed in the cells once suitable transfection strategies are identified. The adenovirus gene transfer

system is also described as suitable for transforming tongue precursor cells (page 33, line 7).

A skilled artisan would be able to use the cells in toxicity testing, testing safety efficacy of a drug, testing efficacy of a drug, developing derivative cell lines and isolating genes or proteins involved in cell differentiation. Suitable assays are well known in the art. Examples of toxicity testing and drug development testing are described in the application on pages 10-11. Other assays that would be clear to a skilled artisan include assays for the identification of drugs or genes involved in causing differentiation of oligodendrocytes, astrocytes, or neurons. For example, precursor cells could be isolated from animals expressing marker genes from neural promoters that are specific to neurons (Tal a-tubulin), oligodendrocytes (myelin basic protein) or astrocytes (glial fibrillary acidic protein). These precursors could then be exposed to exogenous molecules (drugs) and the induction of these cell-type specific markers assayed. Alternatively, the precursors could be transfected with molecules using, for example, adenovirus vectors as described in this application, and the induction of these same cell-type specific markers assayed. Similarly, precursor cells could be isolated from other mammals, including humans, and expression of these markers could be assayed using RT-PCR-based approaches or antibody-based approaches.

A second example involves testing drugs for toxicity. Precursor cells could be isolated from mammals, including humans, passaged and plated into 96-well plates, and then exposed to drugs or natural products. Toxicity would be measured using standard survival assays such as MTT assays, which measure mitochondrial function. Drugs or natural products thought to promote proliferation of precursor cells

or their survival could be assayed using similar approaches and assays.

One skilled in the art, would be able to adapt the standard tests and assays described in the application and known in the art for use with the olfactory precursor cells. Only routine experimentation would be required to use the precursor cells in toxicity testing, testing safety efficacy of a drug, testing efficacy of a drug, developing derivative cell lines and isolating genes or proteins involved in cell differentiation.

Applicants request reconsideration and withdrawal of all the Examiner's rejections and allowance of the claims in this application.

Applicants believe no additional claim fee is required.

Enclosed herewith is a Petition for extending for one month the time to respond to the Examiner's Action. The Commissioner is hereby authorized to charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 19-5425.

Respectfully submitted,

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